Effects of group size and repeated social disruption on the serotonergic and dopaminergic systems in two genetic lines of White Leghorn laying hens

H.-W. Cheng*[1] and A. Fahey†

*Livestock Behavior Research Unit, USDA-Agricultural Research Service, West Lafayette, IN 47907; and †Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

ABSTRACT Farm practices such as increasing group size (GS) and mixing unfamiliar chickens may result in repeated social disruption (RSD) and affect the well-being of hens. To examine whether there are genetic differences in response to social stress, 2 genetic strains of White Leghorn hens were used [i.e., high group production and survivability (HGPS) and DeKalb XL commercial strain (DXL). At 50 wk of age, social stress was created by increasing GS from 4 hens (control) to 8 hens (experimental) per cage and removing hens within the stressed groups to create 4 treatments (control-HGPS, control-DXL, GS/RSD-HGPS, and GS/RSD-DXL). For RSD, 2 hens per cage were moved weekly among the experimental cages within the same treatment. At 58 wk of age, blood sample and brain were collected from 1 hen per cage (n = 10 per treatment). Whole-blood tryptophan and serotonin (5-HT) and plasma norepinephrine (NE), epinephrine (EP), and dopamine (DA) were analyzed by HPLC. The raphe nuclei and the hypothalamus (HYP) were dissected and analyzed by HPLC for the central NE, EP, DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid, 5-hydroxyindoleacetic acid (5-HIAA), 5-HT, and the ratios of DOPAC:DA and 5-HIAA:5-HT. There were no line differences in the concentrations of peripheral tryptophan, 5-HT, EP, NE, and DA in response to GS-RSD ($P > 0.10$). However, neuronal transmitters were regulated differently in the different central nuclei between the lines. In the raphe nuclei, control-HGPS tended to have a higher 5-HIAA:5-HT ratio than the control-DXL ($P = 0.09$). Concentrations of EP were increased in the DXL hens ($P < 0.01$), whereas the HGPS hens had decreased levels of DOPAC ($P < 0.05$) and DA turnover (DOPAC:DA, $P < 0.01$) post GS-RSD. In the HYP, compared with relative controls, there were no significant differences in the concentrations of 5-HT, whereas the levels of 5-HIAA were reduced ($P < 0.01$) after GS-RSD, suggesting that GS-RSD led to a lower 5-HT turnover in the HYP. The results indicate that selection for docility and productivity alters serotonergic and catecholamine homeostasis in hens in response to social stress, GS-RSD.

Key words: group size, repeated social disruption, serotonin, dopamine, laying hen

2009 Poultry Science 88:2018–2025
doi:10.3382/ps.2008-00302

INTRODUCTION

Group size (**GS**) and repeated social disruption (**RSD**) may be more relevant to modern intensified agricultural systems than other types of stressors because animals are often kept in a large group and mixed between different groups depending on their stage of life cycle (Ladewig, 2000). For example, in the United States, laying hens such as W-36 and W-98 may be mixed with unfamiliar conspecifics when they are transferred from the growing facility to the laying facility and housed from 5 to 10 hens per cage. Gross and Siegel (1980) reported that chickens raised in a small stable group are under relatively low social stress, such that 4 hens per cage produce more eggs and have lower plasma corticosterone levels compared with hens housed in a crowded environment, such as 5 or more hens per cage (Mashaly et al., 1984; Cunningham et al., 1988). Anthony et al. (1988) also showed that intermingling unfamiliar White Leghorn hens can be stressful, and the inability of unfamiliar hens to adapt to their social environment results in a greater susceptibility to environmental stimulations and increases the frequency of abnormal behaviors, such as feather pecking, aggression, and cannibalism (Guhl and Allee, 1944; Choudary and Craig, 1972; Bilcík and Keeling, 2000; El-Lethey et al., 2000; Cloutier and Newberry, 2002), and as a result compromises the well-being of the hens.

Neurohormonal systems, such as the sympathetic-adrenal-medullary (**SAM**) axis, control the physiological homeostasis and the coping ability to stimulation of an

Received July 23, 2008.
Accepted June 23, 2009.
[1]Corresponding author: hwcheng@purdue.edu

animal (McCarty, 1995; Moncek et al., 2003; Schommer et al., 2003). A previous study has shown that RSD can increase peripheral catecholamine production (Kvetnansky, 1973). However, more recent studies in mice disagree with this, suggesting that exposure of repetitive chronic stressors may reduce the SAM axis response to stimulations (Davis et al., 1995). Stress-associated alterations of the functions of the SAM axis lead to changes of the serotonergic and dopaminergic systems, resulting in abnormal behaviors and increased aggression in mice (Nikulina and Kapralova, 1992; Lewis et al., 1994). These studies indicate that stressor-associated responses of the SAM axis are dependent on species, environment, and their interaction. The raphe nuclei (**RN**) and the hypothalamus (**HYP**) will be used to evaluate the role of the central nervous system in relation to RSD. The RN is the main source of serotonergic neurons in the brain and is connected to the HYP by ascending projections (Petrovicky et al., 1981); the RN has been studied previously in relation to social stress in rodents (Gardner et al., 2005; Miura et al., 2008). The HYP receives information from the cerebral cortex and the limbic structures and contains numerous neurotransmitters, including norepinephrine (**NE**), dopamine (**DA**), and serotonin (**5-HT**). It serves as a center to coordinate endocrine, autonomic, and somatic motor reactions into appropriate behavior in response to internal and external stimulations (Brodal, 1998).

To study the effects of genetics, environment, and their interactions on hen well-being, a strain of White Leghorn hens was selected for high group productivity and survivability (**HGPS**) using a selection program called group selection (Craig and Muir, 1996a,b; Muir and Craig, 1998; Cheng et al., 2001a,b). Group productivity was based on an average rate of lay, whereas survivability was based on days of survival in colony cages. Hens were not beak-trimmed and high light intensity was used to provide conditions that allowed expression of aggressive behavior, resulting in stress and affecting productivity (Craig and Muir, 1996a,b). The selected line was developed by crossing all available commercial strains in 1982, one of which was the DeKalb XL (**DXL**) commercial strain. Compared with the DXL hens, the HGPS hens had a better rate of lay, feather score, and reduced flightiness and cannibalism (Craig and Muir, 1996a,b). In addition, HGPS hens were more tolerant of heat and cold stress as indicated by lower mortality and greater egg production compared with the DXL hens after environmental stimulations (Hester et al., 1996).

Line differences in production and stress response could be related to the genetic basis of variations in regulating neurohormonal systems. Previous studies have shown that RSD affects the functions of the serotonergic and dopaminergic systems in rodents (Konarska et al., 1990a; Mabry et al., 1995). Those changes influence the stress response and well-being of rodents (Konarska et al., 1990b; Tannenbaum and Anisman, 2003). However, there are few studies about RSD effects on stress response of birds, although the neuroendocrine systems of rodents and birds are analogous (Harvey et al., 1984). The objective of this study was to determine the genetic basis of variations of serotonergic and dopaminergic systems of hens in response to social stress [i.e., an increased GS combined with an unstable environmental stimulation (GS-RSD)].

MATERIALS AND METHODS

Genetic Strain

The 11th generations of hens from the HGPS and DXL lines were used in this study. The HGPS strain was produced from hens selected for high group egg production and survivability, resulting from reduced aggression and cannibalism (Muir, 1996; Muir and Craig, 1998). The DXL line was selected based on individual productivity and is known to have high mortality rates, resulting from aggression and cannibalism in group-housing situations (Craig and Muir, 1996a,b). The differences in productivity and survivability of the 2 strains have been reported previously (Craig and Muir, 1996a,b; Fahey and Cheng, 2008).

Bird Husbandry

The chicks were transferred to the grower house at Purdue Poultry Research Farm on d 1 posthatching. Beak trimming was not performed on the hens at any point in the experiment. The chicks (12 per cage) from the same line were housed in cages with a size at 60.96 cm × 60.96 cm × 45.72 cm (length × width × height; 309.68 cm^2 of floor space/hen) and 2 watering nipples per cage. Industry standard grower feed and water were provided ad libitum throughout the experiment. The room temperature was at 32 to 33°C from d 1 to 3 and then was gradually reduced to 21°C at 36 d of age. Overhead lights were on for 22 h from 0200 to 0000 h daily for the first week, and then on for 10 h from 0600 to 1600 h at 8 wk of age and this was held constant until 17 wk of age.

At 17 wk of age, 240 hens (120 hens from each line) were moved from the growing facility to the laying facility. The overhead lighting was 16L:8D. The hens received an industry standard layer diet (trough space = 30.46 cm/bird) and water (2 water nipples per bird) ad libitum.

Experimental Design

Chickens raised in a small stable group are under relatively low social stress (Gross and Siegel, 1980), such that 4 chickens per cage produce more eggs and have lower plasma corticosterone concentrations compared with chickens housed in a group environment of 5 chickens or more per cage (Mashaly et al., 1984; Cunningham et al., 1988). Based on the findings of these previous studies, RSD was used as a stressor. Because of the

experimental design, the effects of RSD and GS cannot be isolated. The hens subjected to the RSD were housed in 8-hen cages (542 cm^2/birds), whereas control hens were kept in 4-hen cages (542 cm^2/birds) to provide 4 treatments (control-HGPS, control-DXL, GS/RSD-HGPS, and GS/RSD-DXL, 10 cages per treatment). Hens were randomly assigned to the treatments and randomly distributed around the laying room. At 50 wk of age, colored leg bands were put on all hens to identify hens to be moved. The hens of the unstressed group (controls) remained in the same cage for the duration of the experiment. In the GS-RSD group, the hens were marked randomly with different color leg bands. The 2 hens with the same color from the same cage were moved together to another cage in the same treatment once a week. In turn, these hens were replaced in the original cage by 2 hens from another cage in the same treatment. All hens were moved an equal number of times and this was repeated until hens were 58 wk of age as described by Fahey and Cheng (2008).

In our study, chicken care guidelines with the exception of stocking density and cage size were in accordance with the rules and regulations set by the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Craig et al., 1999). The experimental protocol was approved by the Institutional Animal Care and Use Committee at Purdue University (protocol number 00-008-03).

Blood Collection

At the end of the 8-wk period, a 15-mL blood sample was randomly collected from 1 hen per cage (n = 10 cages) by cardiac puncture within 2 min of the hens being taken from their cages. The heparinized blood samples were stored on ice and then transported from the poultry farm to the laboratory facility. A sample of whole blood (300 μL) was transferred to a small test tube and frozen at −80°C for 5-HT and tryptophan (**TRY**) analysis. The remaining sample was centrifuged at 700 × *g* for 15 min at 4°C. The plasma was separated and frozen at −80°C for analysis of peripheral catecholamines [NE, epinephrine (**EP**), and DA].

Brain Collection

The hens were killed by cervical dislocation, and the brains were removed at the end of the experiment. The RN and the HYP were dissected out from each brain and stored at −80°C for analysis of the central concentrations of NE, EP, DA, dihydroxyphenylacetic acid (**DOPAC**), homovanillic acid (**HVA**), 5-hydroxyindoleacetic acid (**5-HIAA**), and 5-HT.

HPLC–Whole-Blood 5-HT and TRY

Whole-blood samples (100 μL/per sample) were acidified in duplicate using 4 *M* perchloric acid (GFS Chemicals, Columbus, OH) and freshly prepared 3% ascorbic acid (Mallinckrodt Chemicals, Philipsburg, NJ). After centrifugation, the supernatant was filtered through a 0.22-um syringe filter and then injected through a column (4.6 mm × 250 mm; part no. 32246, Alltech, Lexington, KY) of the CoulArray HPLC system (ESA Inc., Chelmsford, MA) automatically. The mobile phase flow rate was 1.2 mL/min under 150 bars of pressure. The concentration of 5-HT and TRY was calculated from a reference curve made using standard 5-HT and presented in nanograms per milliliter (Cheng et al., 2001a).

HPLC Assay–Plasma Catecholamines

Duplicate plasma catecholamine samples (NE, EP, and DA) were analyzed using an ESA plasma catecholamine analysis kit (ESA Inc.; Cheng et al., 2001b) using the method outlined by Cheng et al. (2003). Briefly, plasma samples were acidified and deproteinized with 4 *M* perchloric acid. After centrifugation, the acid supernatants and internal standard dihydroxybenzylamine were added and absorbed onto an alumina minicolumn to bind the DA. The columns were then rinsed and eluted with the solutions supplied by the company (ESA Inc.). After injection of eluents into the reverse-phase columns, catechols were detected with Coulochem II electrochemical detectors (ESA Inc.) by liquid chromatography. The mobile phase (75 m*M* Na_2HPO_4, 1.7 m*M* octanesulfonic acid, 25 μ*M* EDTA, 10% CH_3CN, and 100 μL/L triethylamine, adjusted to pH 3.00 with phosphoric acid) flow rate was 1.3 mL/min. Norepinephrine, EP, and DA concentrations were calculated from a reference curve made using supplied standards and were presented in nanograms per milliliter.

HPLC Assay–Brain Samples

The RN and HYP were weighed and 10 μL of 0.2 *M* of double-distilled perchloric acid (GFS Chemicals) was added for every 1 mg of brain tissue. The brain tissues were then homogenized using a tissue tearor (Dremel, Racine, WI), which was cleaned using isopropyl alcohol and ultrapure water between samples. Homogenized brain tissues were centrifuged at 15,400 × *g* for 30 min at 4°C. Five hundred microliters of the brain tissue supernatant was added to 500 μL of MD-TM mobile phase (ESA Inc.) and then vortexed for 1 min, and 300 μL of this solution was filtered using a 0.2-μm polyvinylidene fluoride filter into an HPLC sample tube. The filtered solution was injected into a silica-based column (4.6 mm × 250 mm; part no. 235329, Beckman Coulter, Fullerton, CA). A mobile phase flow rate of 1.3 mL/min was used for 5-HT, 5-HIAA, and catecholamines (EP, NE, DOPAC, HVA, and DA). The concentrations of each compound were calculated from a reference curve made using supplied standards and were presented in nanograms per milliliter.

Table 1. The effects of social stress on blood serotonin and tryptophan in laying hens[1,2]

	DXL		HGPS	
Item	Control	GS-RSD	Control	GS-RSD
5-HT (ng/mL)	17.61 (1.71)	16.10 (2.07)	16.77 (1.70)	19.26 (1.85)
TRY (ng/mL)	10.81 (0.60)	11.38 (0.68)	10.45 (0.59)	11.29 (0.61)
5-HT/TRY	1.67 (0.18)	1.45 (0.21)	1.62 (0.18)	1.73 (0.19)

[1]Data are expressed as means (±SE).

[2]DXL = DeKalb XL (a commercial line); GS-RSD = increased group size-repeated social disruption; HGPS = high group production and survivability (a selected line); 5-HT = serotonin; TRY = tryptophan; 5-HT/TRY = conversion of tryptophan to serotonin.

Statistical Analysis

The analysis cage (1 hen was sampled per cage) was considered the experimental unit. All analysis was conducted using the SAS V9.1 software package (SAS Institute, 2006). Line, treatment, and line × treatment were treated as fixed effects and line and treatment within cage were considered as a random effect. Diagnostic tests were run to determine if data had a normal distribution. Data that did not approach a normal distribution were transformed using Box-Cox transformations as described by Fahey et al. (2007). Log transformations were required for HYP concentrations of EP, DOPAC, HT, and plasma and RN concentrations of DA. Hypothalamus concentrations of 5-HIAA, HVA, and DA turnover and plasma levels of NE and EP were transformed by raising these variables to the power of 0.25. Hypothalamus 5-HT turnover and RN levels of DOPAC, 5-HT turnover, and DA turnover were raised to the power of −0.25. Hypothalamus DA and whole-blood 5-HT were transformed by raising them to the power of −0.50. Finally, plasma EP required a square-root transformation. Comparisons were made between genetic lines within treatment and within genetic lines across treatment. Data presented in this paper show the nontransformed values of the data; however, all *P*-values were calculated using the corresponding transformed data. A Tukey-Kramer adjustment was used to account for multiple comparisons. Significant statistical differences were reported when $P < 0.05$, and statistical trends were reported when $0.05 < P < 0.10$.

RESULTS

Peripheral and Central Serotonergic Systems

There were no significant effects of genetic and genetic-environmental interactions on blood concentrations of 5-HT, TRY, and the conversion of TRY to 5-HT (5-HT/TRY, Table 1). In the RN, there were no significant genetic or genetic-environmental interactions for 5-HT and 5-HIAA. However, 5-HT turnover (5-HIAA:5-HT) tended to be higher in control-HGPS than those of the control-DXL ($F_{2,33} = 2.21$, $P = 0.09$, Table 2). In the HYP, compared with its respective control, a tendency for reduced levels of 5-HT was found in the stressed hens of the HGPS line only ($F_{2,32} = 2.38$, $P = 0.09$), whereas a stress-associated decrease of 5-HIAA concentrations was found in both DXL ($F_{2,33} = 5.00$, $P < 0.01$) and HGPS hens ($F_{2,33} = 4.92$, $P < 0.01$).

HPLC Assay–Plasma Catecholamines

There was no evidence of genetic or genetic-environmental effects on plasma NE, EP, and DA levels, or

Table 2. The effects of social stress on serotonin and 5-hydroxyindoleacetic acid in the raphe nuclei and the hypothalamus of laying hens[1,2]

	DXL		HGPS	
Item	Control	GS-RSD	Control	GS-RSD
Raphe nuclei				
5-HT (ng/mL)	236.51 (13.74)	213.80 (16.83)	206.60 (13.74)	201.45 (15.05)
5-HIAA (ng/mL)	58.63 (5.21)	54.85 (5.88)	63.16 (5.06)	47.94 (5.26)
5-HIAA:5-HT	0.24^{A} (0.02)	0.27 (0.03)	0.33^{B} (0.02)	0.25 (0.03)
Hypothalamus				
5-HT (ng/mL)	317.45 (31.10)	228.80 (33.87)	338.18^{a} (31.10)	222.71^{b} (30.30)
5-HIAA (ng/mL)	79.04^{c} (6.43)	34.39^{d} (6.84)	75.92^{c} (6.43)	38.41^{d} (6.11)
5-HIAA:5-HT	0.27^{a} (0.03)	0.18^{b} (0.03)	0.25^{a} (0.03)	0.17^{b} (0.03)

[a,b; c,d]$P < 0.10$ and $P < 0.05$ within genetic line, respectively.

[A,B]$P < 0.10$ across genetic lines.

[1]Data are expressed as means (±SE).

[2]DXL = DeKalb XL (a commercial line); GS-RSD = increased group size-repeated social disruption; HGPS = high group production and survivability (a selected line); 5-HT = serotonin, 5-HIAA = 5-hydroxyindoleacetic acid; 5-HIAA:5-HT = serotonin turnover.

Table 3. The effects of social stress on blood catecholamines in laying hens[1,2]

	DXL		HGPS	
Item	Control	GS-RSD	Control	GS-RSD
NE (ng/mL)	2.19 (0.72)	2.54 (0.84)	2.83 (0.69)	2.29 (0.75)
EP (ng/mL)	11.06 (3.86)	14.10 (4.52)	15.98 (3.69)	14.87 (4.04)
EP/NE	6.02 (0.81)	5.10 (0.95)	6.57 (0.77)	6.32 (0.85)
DA (ng/mL)	1.60 (0.52)	0.61 (0.60)	1.39 (0.57)	1.82 (0.53)

[1]Data are expressed as means (±SE).

[2]DXL = DeKalb XL (a commercial line); GS-RSD = increased group size-repeated social disruption; HGPS = high group production and survivability (a selected line); NE = norepinephrine; EP = epinephrine; EP/NE = conversion of NE to EP; DA = dopamine.

the conversion of NE and EP to DA (DA/[NE + EP]) in both DXL and HGPS hens after GS-RSD stimulation compared with its relative controls (Table 3, $P > 0.10$).

HPLC Assay–Brain Catecholamines

Analysis of catecholamines in the RN showed that there were no genetic or genetic-environmental interactions for DXL and HGPS hens for NE, DA, or HVA ($P > 0.10$; Table 4). However, the concentrations of EP were increased in the DXL hens after GS-RSD compared with the DXL controls ($F_{2,33} = 2.45$, $P < 0.05$). In the HGPS line, compared with the unstressed HGPS controls, DOPAC concentrations were decreased after stress ($F_{2,38} = 3.25$, $P < 0.05$). Within genetic line, stress had no effect on DA turnover (DOPAC:DA) in the DXL hens, but DA turnover was reduced in the HGPS hens post social GS-RSD ($P < 0.01$). In the HYP, there were no differences in the concentrations of NE, EP, DA, DOPAC, and HVA between the stressed and unstressed hens from both the DXL and HGPS lines ($P > 0.10$; Table 4). However, in the HYP, there was a tendency for reduced DA turnover in DXL hens after GS-RSD compared with the DXL hens in the control environment ($F_{2,34} = 2.48$, $P = 0.08$).

DISCUSSION

The results of the present study provide evidence that there are inheritable differences in the functions of serotonergic and dopaminergic systems in hens in response to social stress, such as GS-RSD.

Tryptophan is an essential amino acid that is a precursor to 5-HT. Peripheral TRY affects the brain 5-HT concentrations by crossing the brain blood barrier (Hamon et al., 1981). Serotonin has multiple functions in controlling an organism's biological processes. In the central nervous system, 5-HT has been involved in the modulation of aggression, regulation of feeding and sexual behaviors, and modulation of stress responses, including social and environmental adaptability (Cook et al., 1995; Olivier et al., 1998). In the peripheral systems, however, biological roles of 5-HT in stress regulation are unclear. Decreases, increases, and

Table 4. The effects of social stress on the catecholamines in the raphe nuclei and the hypothalamus of laying hens[1,2]

	DXL		HGPS	
Item	Control	GS-RSD	Control	GS-RSD
Raphe nucleus				
NE (ng/mL)	69.82 (5.66)	79.75 (6.22)	73.31 (5.45)	78.12 (5.56)
EP (ng/mL)	10.34[c] (1.54)	17.50[d] (1.71)	12.52 (1.57)	16.05 (1.53)
DA (ng/mL)	4.17 (0.54)	4.42 (0.66)	4.03 (0.54)	5.26 (0.59)
DOPAC (ng/mL)	1.54 (0.0.28)	1.02 (0.34)	2.05[e] (0.28)	0.92[d] (0.31)
HVA (ng/mL)	4.67 (0.40)	4.68 (0.44)	5.40 (0.38)	4.82 (0.39)
DOPAC:DA	0.40 (0.07)	0.26 (0.08)	0.53[e] (0.07)	0.18[f] (0.07)
Hypothalamus				
NE (ng/mL)	157.14 (18.50)	201.28 (20.68)	168.96 (18.50)	156.66 (18.50)
EP (ng/mL)	30.99 (5.13)	40.66 (5.73)	38.69 (5.13)	23.54 (5.13)
DA (ng/mL)	13.68 (6.98)	27.29 (7.80)	23.35 (6.98)	23.91 (6.98)
DOPAC (ng/mL)	4.73 (0.89)	3.24 (1.00)	5.59 (0.89)	3.09 (0.89)
HVA (ng/mL)	13.28 (1.94)	16.33 (2.13)	13.89 (1.94)	14.01 (1.91)
DOPAC:DA	0.60[a] (0.17)	0.17[b] (0.18)	0.36 (0.17)	0.19 (0.16)

[a,b; c,d; e,f]$P < 0.10$, $P < 0.05$, and $P < 0.01$ within genetic lines, respectively.

[1]Data are expressed as means (±SE).

[2]DXL = DeKalb XL (a commercial line); GS-RSD = increased group size-repeated social disruption; HGPS = high group production and survivability (a selected line); NE = norepinephrine; EP = epinephrine; DA = dopamine; DOPAC = dihydroxyphenylacetic acid; HVA = homovanillic acid; DOPAC:DA = DA turnover.

no changes in 5-HT concentrations have been associated with various abnormal behaviors. The conflicting data from different investigations could be related to different genetic selection programs, species, behavioral evaluation, and stressors used as well as duration and frequency of stressor presentation.

In the present study, the data showed that there were no significant effects of genetic and genetic-environmental interactions on blood concentrations of 5-HT, TRY, and the conversion of TRY to 5-HT. Similar findings were also shown in the RN. In the RN, there were no significant differences in the examined neurotransmitters and their metabolites between the lines. In the HYP, compared with its respective controls, a tendency for reduced levels of 5-HT was found in the stressed hens of HGPS line only, whereas a stress-associated decrease of 5-HIAA concentrations was found in both DXL and HGPS hens. These results suggest that the serotonergic system is regulated differently between periphery and the brain in response to stimulations, which is genetic-environment dependent.

Similarly to our findings, previous studies have shown that external stimulations reduce 5-HTconcentrations in the HYP in rodents after stressful events such as electric foot shock treatment (Malyszko et al., 1994), forced swim (Briones-Aranda et al., 2005), and maternal separation (Arborelius and Eklund, 2007).

A possible reason for the lack of effect of GS-RSD on 5-HT in the RN of the DXL and HGPS hens could be related to the anatomical distribution of serotonergic system. Serotonin is synthesized in the RN and then transported along axonal terminal pathways to various parts of the brain including the HYP and is associated with different behaviors. Metabolism of 5-HT is mostly conducted in the targeted nuclei, including the HYP. The current results suggest that 5-HT and 5-HIAA in the RN may not be suitable biomarkers for the GS-RSD stimulation.

There was no evidence of genetic or genetic-environmental effects on plasma NE, EP, and DA levels, or the conversion of NE and EP to DA in both DXL and HGPS hens after GS-RSD stimulation compared with their conspecific controls. Similarly, Korte et al. (1997) reported no differences in the levels of NE and EP in the birds divergently selected for high and low feather pecking. However, when restrained, the hens selected for high feather pecking had elevated EP. These data further indicate that stress response of hens is gene- and stressor-dependent. The stressor (GS-RSD) used in the study may not be stressful enough to the hens or the hens may have adapted to the repeated stimulations. Davis et al. (1995) evidenced that the SAM axis responses can be reduced by repetitive exposure to chronic stressors.

The current data showed that in the RN, there were no genetic or genetic-environmental interactions for DXL and HGPS hens for NE, DA, or HVA. However, in the RN, compared with the relative controls, stress-associated increases in concentrations of EP were found in the DXL, whereas stressed HGPS hens had reduced DOPAC concentrations, resulting of a decrease of DA turnover (DOPAC:DA) in the HGPS hens but not in the DXL hens. In the HYP, there were no differences in the concentrations of NE, EP, DA, DOPAC, and HVA between the stressed and unstressed hens from both the DXL and HGPS lines. However, there was a tendency for reduced DA turnover in DXL hens after GS-RSD stimulation compared with those from the DXL line. Similar to the present results, Funada and Hara (2001) and Miura et al. (2002) reported that there was no change in DA concentrations in the RN of rats after psychological stress created by using a communication box paradigm and social isolation. The present and previous findings indicated that genetic variations of animal stress responses are stressor- and brain region-dependent.

Although it is unclear what mechanisms underlie the different regulation of catecholamines in the blood and brain tissues in the presently selected hens, previous studies have reported that such differences could be induced by a stress-related unbalance of activities of enzymes involved in catecholamine metabolism, such as DA-β-hydroxylase (**DβH**; Kuchel et al., 1987). The DβH enzyme converts DA to NE, which is converted to EP by phenylethanolamine N-methyltransferase. Without detecting DβH activities in the present study, we cannot assume that DβH activity was regulated differently between the DXL and HGPS hens after GS-RSD. However, previous research showed that DβH activity increased in brain tissue after chronically repeated cold stress and ethanol stress in rats (Daiguji et al., 1982; Patterson-Buckendahl et al., 2005). There is evidence to suggest that there are hereditary-based stress triggers that differently regulated gene expressions of catecholamine biosynthetic enzymes in the adrenal medulla (Nankova et al., 1999). Further studies should be conducted to test this hypothesis in the current lines of hens.

In conclusion, the results indicate that there was a genetic basis of variation in metabolism of serotonergic and dopaminergic systems in the brains between the present lines after GS-RSD stimulation. In the RN, compared with HGPS hens, DXL hens tended to have lower baseline levels of 5-HT turnover and increased EP concentrations after stress stimulation. These changes may be associated with their abnormal and aggressive behaviors reported previously. These results suggest that different selection strategies (group selection vs. individual selection) have altered neuroendocrine stress responses of hens, resulting in the unique characteristics of the line in productivity and survivability, along with different coping strategies for various environmental stressors. The data provide important information that could be used to improve the welfare of domestic hens selected for egg production and to prepare hens for induced molting.

ACKNOWLEDGMENTS

We are grateful to students and employees of the USDA-Agricultural Research Service Livestock Behavioral Research Unit and the Purdue University poultry farm for their assistance in the collection of data. The authors also express gratitude to Don Lay of the Livestock Behavior Research Unit and the 2 anonymous reviewers for improving the quality of the paper. This work was supported by a USDA-National Research Initiative grant.

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